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Protocol-BODIPY FL C5-Sphingomyelin or BODIPY FL C5-Ceramide staining in mESCs

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An abbreviated version of this protocol was published in eLIFE in May 2021

SIRT1 regulates sphingolipid metabolism and neural differentiation of mouse embryonic stem cells through c-Myc-SMPDL3B

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Detailed protocol

BODIPY FL C5-Sphingomyelin or BODIPY FL C5-Ceramide staining Protocol in mESCs

1. Prepare 1mM stock solution of BODIPY FL C5-Sphingomyelin (N-(4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Pentanoyl) Sphingosy Phosphocholine) (Invitrogen, D3522) (BODIPY-SM) or BODIPY FL C5-Ceramide (N-(4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-Pentanoyl)Sphingosine) (Invitrogen, D3521) in the mixed solvent of chloroform:ethanol (19:1 v/v).
2. Dispense 50 µl of this BODIPY-SM stock solution from step 1 into a small glass test tube and dry, first under a stream of nitrogen, and then under vacuum for at least 1 hour. After the liquid phase has been completely dried off, add 200 µl of absolute ethanol into the test tube to redissolve the dried BODIPY-SM.
3. Prepare 5µM fatty acid-free BSA (Roche, 03117057001) solution by using 10 ml Hanks' buffered salt solution (Gibco, 14175-095) containing 10 mM HEPES (Gibco, 15630-106) (HBSS/HEPES buffer pH 7.4).
4. Add the prepared 200 µl BODIPY-SM solution in ethanol from step 2 into the 10 ml of 5µM fatty acid-free BSA solution and mix properly on a vortex mixer to generate the working solution (5 µM BODIPY-SM + 5 µM BSA) which can be store at -20°C.
5. Grow the mESCs or E14 cells on 0.1% gelatin coated Nunc^{TM} Glass Base Dish (Thermo Scientific 150682) at the density of approximately 5x10^{4} cells/cm^{2}.
6. For staining, remove all growth medium and wash cells 3 times with HBSS/HEPES buffer. Apply sufficient working solution from step 4 to completely cover all cells and incubate cells at 4°C for 30 minutes (protect from light).
7. Completely remove all working solution and wash cells 3 times with ice-cold growth medium. After wash, add adequate fresh cell growth medium and incubate cells at 37°C for 30 minutes (protect from light).
8. After incubation, wash cells with fresh medium (room temperature). Those stained cells are directly examined on Glass Base Dish and images were captured by Zeiss LSM 780 UV confocal microscope (Abs 505 nm and Em 511 nm).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fan, W. and Li, X. (2021). Protocol-BODIPY FL C5-Sphingomyelin or BODIPY FL C5-Ceramide staining in mESCs. Bio-protocol Preprint. bio-protocol.org/prep1270.
2. Fan, W., Tang, S., Fan, X., Fang, Y., Xu, X., Li, L., Xu, J., Li, J., Wang, Z. and Li, X.(2021). SIRT1 regulates sphingolipid metabolism and neural differentiation of mouse embryonic stem cells through c-Myc-SMPDL3B. eLIFE. DOI: 10.7554/eLife.67452